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A Delay in the *Saccharomyces cerevisiae* Cell Cycle That Is Induced by a Dicentric Chromosome and Dependent upon Mitotic Checkpoints

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Dicentric chromosomes are genetically unstable and depress the rate of cell division in *Saccharomyces cerevisiae*. We have characterized the effects of a conditionally dicentric chromosome on the cell division cycle by using microscopy, flow cytometry, and an assay for histone H1 kinase activity. Activating the dicentric chromosome induced a delay in the cell cycle after DNA replication and before anaphase. The delay occurred in the absence of *RAD9*, a gene required to arrest cell division in response to DNA damage. The rate of dicentric chromosome loss, however, was elevated in the *rad9* mutant. A mutation in *BUB2*, a gene required for arrest of cell division in response to loss of microtubule function, diminished the delay. Both *RAD9* and *BUB2* appear to be involved in the cellular response to a dicentric chromosome, since the conditionally dicentric *rad9 bub2* double mutant was highly inviable. We conclude that a dicentric chromosome results in chromosome breakage and spindle aberrations prior to nuclear division that normally activate mitotic checkpoints, thereby delaying the onset of anaphase.

The fidelity of eukaryotic chromosome segregation requires that sister chromatids be equally partitioned into progeny cells. The centromere is essential for this process because it sponsors the kinetochore, which mediates chromosome attachment to spindle microtubules, and because it ensures that sister chromatids segregate toward opposite spindle poles. The instability of dicentric chromosomes has been described cytologically in classical studies of higher eukaryotes. One possible consequence of a chromosome having two centromeres is the formation of an anaphase bridge, which results when the centromeres of a dicentric chromatid are pulled toward opposite poles (27). This chromatin bridge may break, catalyzing a variety of healing events that result in a breakage-fusion-bridge cycle (28) or monocentric derivatives (29).

The study of dicentric chromosomes in *Saccharomyces cerevisiae* has been facilitated by the cloning of centromere DNA (4, 5, 16). Although cytological constraints preclude the direct observation of chromosomes in *S. cerevisiae* (11, 35), the instability of dicentric chromosomes has been described by both molecular and genetic methods (12, 17, 20, 22, 26). Most studies of dicentric chromosome behavior in *S. cerevisiae* have dealt with the types of rearrangements which resolve the dicentric chromosome into a monocentric derivative. Surprisingly, dicentric chromosomes can be transmitted intact during both meiotic and mitotic cell divisions (12, 22). The cells propagating a dicentric chromosome divide slowly, however (22, 26), and predominantly have large buds and a single nucleus (17). Therefore, in addition to being unstable, a dicentric chromosome may impede cell cycle progression at a late stage.

Hartwell and Weinert have proposed that delays in the cell cycle are mediated by checkpoints which ensure dependency relationships among otherwise independent events (15). Checkpoints have been identified genetically in *S. cerevisiae* by mutations which relieve dependencies, thereby uncoupling cell cycle events. *RAD9*, for example, is a component of a checkpoint that surveys chromosome integrity and arrests the cell during G_2 in response to DNA damage to allow repair prior to the chromosome being segregated (45). *BUB2* (budding uninhibited by benzimidazole) is a component of a checkpoint that maintains the order of events in response to complete loss of microtubule function (18). Recessive mutations in *BUB2* uncouple cell cycle events in the absence of a mitotic spindle, a condition which results in arrest during G_2 in the presence of *BUB2* function (36). Therefore, *RAD9* and *BUB2* are required for coordinating the events of mitosis in response to perturbations which affect chromosome segregation. A dicentric chromosome might elicit a cell cycle delay by activating a mitotic checkpoint(s).

We have used a conditionally dicentric chromosome III (Ch. III), originally described by Hill and Bloom (17), to study the effect of a dicentric chromosome on cell cycle progression. Dicentric Ch. III (Fig. 1) consists of a native *CEN3* sequence and an ectopic *GALCEN3* (2). The second centromere is made conditional by the *GAL1* promoter which directs transcription at element I of *CEN3*. Galactose induces transcription which inactivates the centromere, whereas glucose represses the promoter and allows the centromere to function (16). We studied the effects of the activated dicentric chromosome by using microscopy, flow cytometry, and an assay for histone H1 kinase activity and tested these effects in mutants defective for checkpoint control (*rad9* and *bub2*).

MATERIALS AND METHODS

Yeast strains and transformations. Table 1 lists the relevant genotypes and sources of *S. cerevisiae* strains. Strains were constructed by standard genetic methods (42). *rad9* was scored by sensitivity to a 64.5-krad exposure from a ^{137}Cs source. *bub2* mutant strains were scored by sensitivity to 10 to 15 μg of benomyl (Dragon Corp.) per ml during growth at 23°C. DNA transformations of yeast cells were performed by

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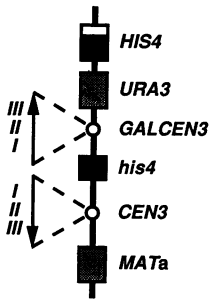

FIG. 1. Diagram of conditionally dicentric Ch. III. A plasmid containing *CEN3* under the functional control of the *GAL1* promoter was integrated at *HIS4* to produce a conditionally dicentric chromosome (17). The ectopic *GALCEN3* is in the inverse orientation with respect to the native *CEN3*, as indicated by the arrows, which represent the order of centromeric elements I, II, and III. This arrangement precludes an intrachromosomal recombination event that would remove one of the two centromeric sequences. *HIS4* sequences are duplicated by the integration event, but recombination between these sequences is precluded by selecting for *URA3*. *MAT***a** and *URA3* are located on opposite sides of the centromeres and serve as markers for measuring dicentric Ch. III loss.

the lithium acetate procedure (19) or by a colony transformation procedure (21). The conditionally dicentric Ch. III (Fig. 1) was constructed in different genetic backgrounds by transforming various yeast strains with pR285-*GALCEN3*#4 DNA (*HIS4*:*GALCEN3*:*URA3*; provided by K. Bloom) which had been cut with *Xho*I and then selecting for transformants on SC − Ura (synthetic complete medium lacking uracil) plates containing galactose. Integration of plasmid pR285-*GALCEN3*#4 was confirmed by gel transfer of genomic DNA and hybridization to a radioactively labeled probe (41). The *RAD9* gene was disrupted by transforming strains with *Bam*HI-cleaved pTW031 DNA (Δ*rad9*:*LEU2*; provided by T. Weinert).

Growth and media. YM-1, YEP, and SC media were as previously described (13, 42). Galactose or glucose was added to a final concentration of 2% in all media. All cultures were grown at 30°C. Cell counts were performed by microscopy with the aid of a hemocytometer.

Microscopy and flow cytometry. Cells were stained with 4,6-diamidino-2-phenylindole (Sigma), viewed by microscopy, and photographed as previously described (32). The DNA content of cells was determined by flow cytometry of propidium iodide-stained cells (3). The quantitative data reported for flow cytometry are ratios of the percentage of cells with a 2n DNA content to the percentage of cells with a 1n content. These data are the average ratios from two independent methods for estimating the areas under the peaks, assuming Gaussian distributions.

Histone H1 kinase assay. The histone H1 kinase activity of cell populations was measured as described previously (23). Cultures of J178-1D#4 and 1108-4 were analyzed after growth to mid-logarithmic phase (10^6 cells per ml) in SC − Ura with galactose and again after 5 h of additional growth in SC − Ura with glucose. Strain 2061-0 was analyzed after growth to mid-logarithmic phase in both YM-1 with glucose and YM-1 with galactose and also after 3 h of growth in the presence of 15 μg of nocodazole per ml in YM-1 with glucose.

Measurement of dicentric Ch. III loss. Loss of the conditionally dicentric Ch. III was scored in diploid strains 1107 and 1135 by screening for loss of both *URA3* and *MAT***a**, markers located on opposite sides of the two centromeres. *URA3* is distal to the ectopic *GALCEN3* at the *HIS4* locus, and *MAT***a** is distal to the native *CEN3* (Fig. 1). Individual clones were picked and grown in SC − Ura containing galactose to 10^6 cells per ml, filtered, and resuspended in SC − Ura containing glucose. After various times (0, 3, and 6 h),

TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype	Source or reference
Haploids		
PT1	*MAT***a** *hom3*	L. Hartwell
PT2	*MAT*α *hom3*	L. Hartwell
2061-0	*MAT***a** *ura3*	M. Johnston
1121-11-1	*MAT***a**	This study
J178-1D#4	*MAT***a** *leu2 ura3 HIS4:GALCEN3:URA3*	17
1108-4	*MAT***a** *leu2 ura3 HIS4:GALCEN3:URA3 rad9::LEU2*	This study
1129-0	*MAT***a** *leu2 ura3 bub2 HIS4:GALCEN3:URA3*	This study
1134-0	*MAT***a** *leu2 ura3 bub2 HIS4:GALCEN3:URA3 rad9::LEU2*	This study
Diploids		
1109	*MAT***a** *leu2* + / *MAT*α + *ura3*	This study
1107	*MAT***a** *leu2 ura3 HIS4:GALCEN3:URA3* / *MAT*α + *ura3* +	This study
1135	*MAT***a** *leu2 ura3 rad9::LEU2 HIS4:GALCEN3:URA3* / *MAT*α *leu2 ura3 rad9::LEU2* +	This study
1149	*MAT***a** *leu2 ura3 bub2 HIS4:GALCEN3:URA3* / *MAT*α *leu2 ura3 bub2* +	This study
1238-1	*MAT***a** *leu2 ura3* / *MAT*α *leu2 ura3*	This study
1239-1	*MAT***a** *leu2 ura3 rad9::LEU2* / *MAT*α *leu2 ura3 rad9::LEU2*	This study

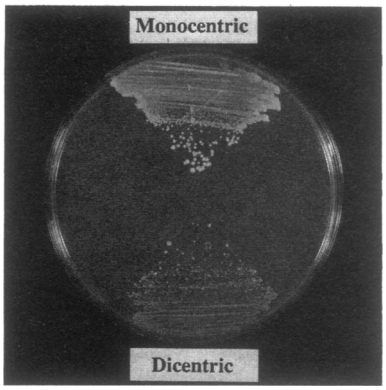

FIG. 2. Colony growth of monocentric and dicentric haploid *S. cerevisiae* strains. Wild type strain 1121-11-1 (monocentric) and conditionally dicentric strain J178-1D#4 (dicentric) were grown in SC − Ura liquid medium with galactose to mid-logarithmic phase, streaked onto an SC − Ura plate containing glucose, and incubated for 3 days.

aliquots were diluted at least 1,000-fold and plated on YEP plus galactose (YEPGal). The frequency of *URA3* loss was measured by replica plating to SC − Ura plates with galactose. Loss of *MAT***a** confers fertility to diploids and was scored by replica plating to YEPGal plates spread with mating-type tester strains (PT1 and PT2) and then selecting for maters on minimal medium containing galactose. The rates of loss were derived from the frequencies obtained at 0 h and 6 h and adjusted for the number of cell divisions that occurred during that time interval.

To estimate the rates of loss for monocentric Ch. III, haploid revertants for each genotype (*RAD9* and *rad9*) that had lost *GALCEN3*::*URA3* and that grew at wild-type rates in glucose-containing medium were isolated and used to construct monocentric Ch. III diploid strains. Fluctuation analysis with 10 clones each of 1238-1 and 1239-1 was used to determine the rate at which fertile diploids were produced, using the method of the median (24) as described by Gerring et al. (9).

Plating efficiency. Haploid strains J178-1D#4, 1108-4, 1129-0, and 1134-4 were grown for 20 h in SC − Ura with galactose, sonicated for 12 bursts at 33 W, counted with the hemocytometer, diluted in sterile H_2O, and plated onto YEPD. Colonies were counted after 2.5 days.

RESULTS

Figure 1 is a diagram of the conditionally dicentric Ch. III. The chromosome is functionally monocentric when the strain is grown with galactose but becomes dicentric when the strain is grown with glucose. When a conditional dicentric haploid strain was grown on agar medium containing glucose, the size of the colonies was heterogeneous (Fig. 2). Most colonies were small and consisted of cells with an intact dicentric Ch. III. A few colonies were large and consisted of cells with a rearranged, monocentric Ch. III (22, 26). Therefore, prolonged growth in the presence of glucose selects for cells carrying monocentric derivatives. A high frequency of chromosomal rearrangement, along with selection for better growth associated with these rearrangements, can obscure the effect on cell division of the dicentric chromosome. We have therefore restricted most of our analyses to the first two doublings after cultures were transferred from galactose- to glucose-containing medium. A haploid dicentric strain (J178-1D#4) doubles twice in 6.8 h after the shift to medium containing glucose, compared with 4.0 h for a wild-type strain (2061-0). The typical experiment was to grow cells to mid-logarithmic phase in SC − Ura medium containing galactose (monocentric) and then transfer the cells to SC − Ura medium containing glucose (dicentric). Aliquots of cells were taken at various times after the transfer to investigate the effect of the dicentric chromosome on the first two cell cycles.

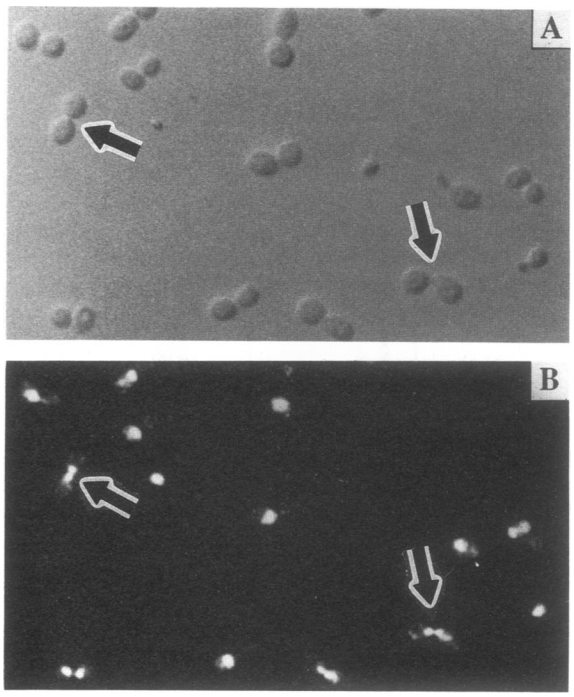

FIG. 3. Morphology of cells propagating a dicentric chromosome. Conditionally dicentric haploid strain J178-1D#4 was grown in SC − Ura liquid medium with galactose to a density of 5×10^6 cells per ml and then transferred to SC − Ura liquid medium with glucose. Cells were fixed after 5 h of growth and stained with 4,6-diamino-2-phenylindole. A and B are photomicrographs of the same field, using differential interference contrast and fluorescence microscopy, respectively. The arrows denote large budded cells having a single bilobed nucleus within the neck of the bud.

The cell cycle delay. The morphologies of cells propagating the dicentric chromosome are shown in Fig. 3. We found that 59% ($n > 100$) of the cells had large buds and a single undivided nucleus (arrows in Fig. 3) when grown in glucose, compared with 30% ($n > 100$) when grown in galactose. These data are similar to those reported previously (2) and suggested that cells with the dicentric chromosome grew slowly because cell division was impeded at a discrete stage. We used flow cytometry of propidium iodide-stained cells to measure cellular DNA content to determine the distribution of cells in the cell cycle. Typically, there are two peaks of fluorescence (Fig. 4, 0 h) corresponding to a 1n (G_1) and 2n (G_2/M) content of DNA for haploids. The data show that the cell cycle distribution of cells with a monocentric chromosome is not altered by the transfer from galactose to glucose. In contrast, the distribution of cells carrying the conditional dicentric chromosome was normal after growth in medium containing galactose (Fig. 4, 0 h) but was shifted toward the 2n peak after the transfer to glucose-containing medium.

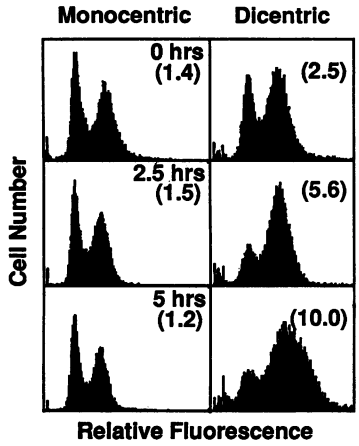

FIG. 4. Flow cytometry for DNA content of monocentric and dicentric strains. Haploid strains 2061-0 (monocentric) and J178-1D#4 (dicentric) were grown in SC − Ura liquid medium with galactose to a density of 5 × 10^6 cells per ml, washed, and transferred to SC − Ura liquid medium with glucose. Aliquots of cells were taken at 0, 2.5, and 5.0 h after the switching to glucose and prepared for flow cytometry. For each graph, cell number = 30,000. Each number in parentheses is the ratio of the percentage of cells in the 2n peak to the percentage of cells in the 1n peak.

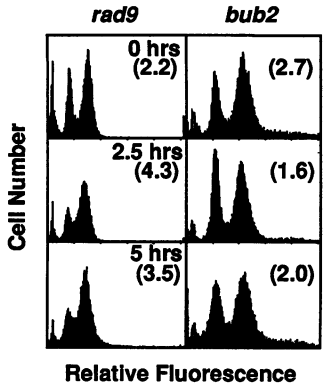

FIG. 5. Flow cytometry for DNA content of dicentric checkpoint mutants. Haploid strains 1108-4 (*rad9*) and 1129-1 (*bub2*) were grown in SC − Ura liquid medium with galactose to a density of 5 × 10^6 cells per ml, washed, and transferred to SC − Ura liquid medium with glucose. Aliquots of cells were taken at 0, 2.5, and 5.0 h after the switch to glucose and prepared for flow cytometry. For each graph, cell number = 30,000. Each number in parentheses is the ratio of the percentage of cells in the 2n peak to the percentage of cells in the 1n peak.

These data suggest that activating the dicentric chromosome induced a delay in the cell cycle after the bulk of DNA was replicated.

The morphology of cells containing the dicentric chromosome (Fig. 3) implies that the cells were delayed prior to the initiation of anaphase. We assayed for histone H1 kinase activity, which serves as a biochemical marker for the onset of anaphase. We compared the histone H1 kinase activities of a conditional dicentric strain grown in galactose and then in glucose-containing media. A threefold greater activity was recovered when conditionally dicentric cultures were grown in the presence of glucose than when they were grown in the presence of galactose, and this elevated activity was similar to the level found in wild-type cells arrested with nocodazole (Table 2). Transferring wild-type cultures from galactose to glucose-containing medium had little effect on the level of histone H1 kinase activity. These data show that the dicentric chromosome delays cells prior to anaphase with a high level of histone H1 kinase activity. Cells confronted with either DNA damage or impaired microtubule function also arrest late in the cell cycle with a high level of histone H1 kinase activity (2a, 23). This cellular response to these two conditions is under the control of the *RAD9*- and *BUB2*-dependent checkpoints respectively (18, 45).

TABLE 2. Histone H1 kinase activity of asynchronous cultures

Growth condition	H1 kinase activity[a]		
	Monocentric (*RAD9*)	Dicentric	
		RAD9	*rad9*
Galactose	6.5 ± 0.3	6.4 ± 0.6	5.8 ± 0.6
Glucose[b]	6.9 ± 0.6	18.5 ± 2.5	12.7 ± 0.9

[a] The following haploid strains were used: 2061-0 (monocentric [*RAD9*] and wild type, J178-1D#4 (dicentric [*RAD9*]), and 1108-4 (dicentric [*rad9*]). Data are expressed as nanomoles of phosphate transferred per 30 min per mg of protein at 37°C and are means ± standard errors of the means of three independent measurements. The activity for the wild type subjected to nocodazole arrest (15 μg/ml at 30°C for 3 h) was 19.3 ± 1.8.

[b] For a 5-h period at 30°C.

Effect of *rad9* on the mitotic delay. Cytological studies in other cell types emphasize the likelihood of dicentric chromosomes to form anaphase bridges and break during mitosis. In *S. cerevisiae*, a broken chromosome activates the *RAD9*-dependent checkpoint which delays the onset of anaphase, presumably to allow time for DNA repair before the chromosome is segregated (45). If the delay induced by dicentric Ch. III is due solely to this chromosome being broken before mitosis, loss of *RAD9* should decrease the doubling time. Haploid strain 1108-4 (*rad9* dicentric), which is isogenic with J178-1D#4 (*RAD9* dicentric), doubled twice in 5.0 h after the transfer from galactose- to glucose-containing medium, compared with 6.8 h to double twice for J178-1D#4. However, the *rad9* strain divided slower than the wild-type strain (4.0 h to double twice), suggesting that an activated *RAD9* checkpoint was not the sole cause of mitotic delay. Flow cytometry of propidium iodide-stained cells (Fig. 5) showed that the *rad9* mutation did not abolish the delay. The results of the histone H1 kinase assay were also consistent with a suppression of the delay by *rad9*. The level of kinase activity for the *rad9* dicentric strain (1108-4) increased twofold compared with the level of a wild-type monocentric strain (2061-0) after a transfer to medium with glucose (Table 2) but was slightly less than in the wild-type dicentric strain (J178-1D#4). These data suggest that a *rad9* mutant still delays in response to the dicentric chromosome but to a lesser extent than does a *RAD9* strain.

We used a chromosome loss assay to determine whether *rad9* affects the transmission of dicentric Ch. III. We constructed diploid strains 1107 (*RAD9/RAD9*) and 1135 (*rad9/rad9*), heterozygous for conditional dicentric Ch. III. This chromosome contains two markers, *URA3* and *MAT***a**, that can be used to monitor the accuracy of its transmission. Chromosome losses were measured after the strains were grown in SC − Ura liquid medium containing either galactose or glucose and then plated onto YEPGal, on which the proportion of cells that were phenotypically both Ura^- (loss of *URA3*) and α maters (loss of *MAT***a**) could be scored. The

dicentric chromosome was lost at a greater rate in the *rad9/rad9* diploid strain (36.1 ± 2.2% per division, $n = 3$) than in the *RAD9/RAD9* diploid strain (11.3 ± 4.8% per division, $n = 5$) during the first two divisions of growth in the presence of glucose. We infer that the *RAD9* checkpoint is normally activated by dicentric chromosome breakage and that the chromosome is lost as a result of unrepaired breaks in the absence of *RAD9* function. We constructed strains carrying a monocentric derivative of Ch. III and recovered α-mating diploids at a rate of 2.42×10^{-5} (*RAD9/rad9*) and 3.42×10^{-5} (*rad9/rad9*). Although these measurements include both loss and mitotic recombination events, they estimate the maximal rate of loss for monocentric Ch. III in these strains. Therefore, a dicentric chromosome is lost at a rate approximately 10^4-fold greater than that of a monocentric chromosome.

Effect of *bub2* on the mitotic delay. The mitotic delay was alleviated but not abolished by *rad9*, suggesting that another mitotic checkpoint was involved. *BUB2* function is necessary to coordinate cell cycle events in mitosis. We constructed a conditionally dicentric *bub2* strain (1129-0) to determine whether the mitotic delay was altered by the *bub2-1* mutation. Thirty percent of dicentric *bub2* cells (1129-0) ($n > 100$) had a large bud and a single, undivided nucleus after growth for 2.5 h on glucose, compared with 59% ($n > 100$) for the wild-type dicentric strain (J178-1D#4). The data from flow cytometry (Fig. 5) show that the distribution of cells in the cell cycle after activation of the dicentric chromosome is different than for the wild-type dicentric population and approximates the profile for cells carrying a monocentric Ch. III (Fig. 4). These results indicate that a *bub2* mutation affects the cellular response to a dicentric chromosome.

Synergistic effect of *rad9* and *bub2* on the mitotic delay. If both *RAD9* and *BUB2* are involved in the response to a dicentric chromosome, then a conditionally dicentric double mutant lacking both checkpoint functions should have a more severe phenotype than does either single mutant. We constructed a *rad9 bub2* dicentric strain (1134-4) and determined the viability on medium containing glucose. Equal-size inocula (approximately 2×10^4 cells) were streaked onto solid medium containing glucose. As shown in Fig. 6, there was a synergistic effect on the viability of the conditionally dicentric double mutant. In contrast, a monocentric *rad9 bub2* mutant exhibited wild-type viability. Colony growth reflects the consequence of cumulative cell divisions with a dicentric chromosome, so our conclusions do not pertain to events occurring in a single cell cycle. These results support earlier findings that both *BUB2* and *RAD9* are involved in the cellular response to a dicentric chromosome.

Effect of dicentric Ch. III in diploids. We found that the frequency of dicentric Ch. III loss was high (1.5 ± 0.9%, $n = 5$) even when diploid strain 1107 was grown in medium containing galactose, a condition that should have repressed the *GALCEN3* centromere. This frequency of loss for dicentric Ch. III is 10^3-fold greater than the frequency of loss in a diploid with a monocentric Ch. III ($8.1 \times 10^{-5} \pm 2.2 \times 10^{-5}$; strain 1238-1). The conditional dicentric diploid strains grew slower in medium containing galactose than did monocentric strains. These observations suggested that the conditional centromere was not completely inactivated in diploid strains by growth in the presence of galactose. Flow cytometry of conditional dicentric diploid strains (Fig. 7) shows that the *RAD9/RAD9* strain 1107 and the *rad9/rad9* strain 1135 have cell cycle distributions consistent with a mitotic delay occurring during growth in medium with galactose. This result is in contrast to the data for haploids (Fig. 4 and 5), in which case the distributions of cells from *RAD9* and *rad9* dicentric strains grown in the presence of galactose approximated the cell cycle distribution of wild-type cells. There are two possible explanations for these results. First, the dicentric chromosome might not have been completely inactivated in diploids by growth in the presence of galactose as it was in haploid strains. Alternatively, the chromosome might have been partially activated in both haploids and diploids during growth in medium with galactose, but diploids might be more sensitive to dicentric chromosome behavior. In either case, the delay in diploids under repressive conditions for the second centromere is mechanistically similar to the delay in

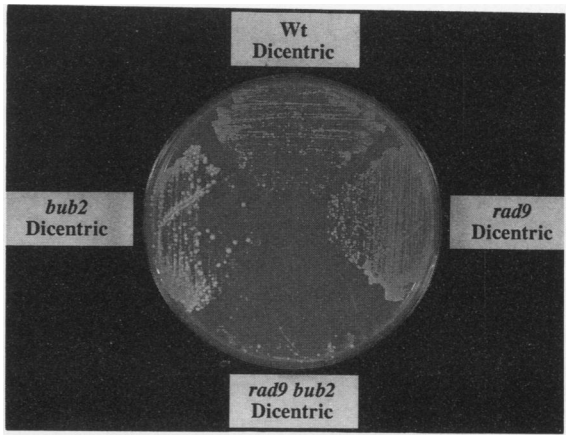

FIG. 6. Growth and viability of dicentric checkpoint mutants. Haploid strains J178-1D#4 (dicentric), 1108-4 (*rad9* dicentric), 1129-2 (*bub2* dicentric), and 1134-2 (*rad9 bub2* dicentric) were grown in SC − Ura liquid medium with galactose to a density of 5×10^6 cells per ml, streaked onto an SC − Ura plate containing glucose, and incubated for 3 days. The plating efficiencies on YEPD were as follows: dicentric, 11.2% ± 1.2%; *rad9* dicentric, 40.3% ± 5.6%; *bub2* dicentric, 10.8% ± 2.4%; and *rad9 bub2* dicentric, 4.8% ± 1.0% ($n = 3$).

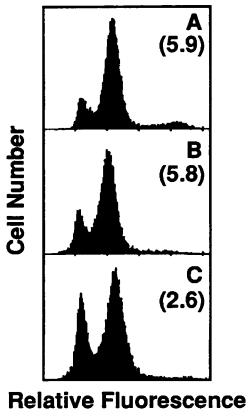

FIG. 7. Flow cytometry for DNA content of dicentric diploid strains. Wild-type dicentric strain 1107 (A), *rad9/rad9* dicentric strain 1135 (B), and *bub2/bub2* dicentric strain 1149 (C) were grown to a density of 5×10^6 cells per ml in SC − Ura with galactose, and samples of cells were prepared for flow cytometry. For each graph, cell number = 30,000. Each number in parentheses is the ratio of the percentage of cells in the 4n peak to the percentage of cells in the 2n peak.

haploids when the dicentric chromosome is activated, since the delay is diminished in a *bub2/bub2* dicentric strain (1149), as shown by flow cytometry (Fig. 7).

DISCUSSION

The mitotic delay induced by a dicentric chromosome. We have characterized the effect of a conditionally dicentric chromosome on cell division in *S. cerevisiae*. Activating the dicentric chromosome elicits a delay of cell division at a discrete stage in the cell cycle, after DNA replication and prior to anaphase. The predominant morphology is a cell with a large bud and an undivided nucleus. Although the stages of mitosis cannot be defined cytologically in *S. cerevisiae*, the high level of histone H1 kinase activity found in populations of wild-type cells carrying a dicentric chromosome is consistent with these cells being delayed near the onset of anaphase. We conclude that *S. cerevisiae* alters the timing of mitosis in response to a dicentric chromosome.

Involvement of mitotic checkpoints. Checkpoints have previously been implicated in the regulation mitosis (15) and are necessary for delaying anaphase in *S. cerevisiae* (18, 25, 45). We have found that two previously identified genes are involved in the mitotic delay elicited by a dicentric chromosome. *RAD9* and *BUB2* are required for cell cycle arrest in response to DNA damage (45) and complete loss of microtubule function (18), respectively; recessive mutations in these genes abolish the ability of a cell to modulate the timing of mitosis in response to specific perturbations, thereby uncoupling events of the cell cycle.

bub2 mutants appear to initiate early cell cycle events in the absence of both nuclear division and cytokinesis when there is complete loss of microtubule function (18). Bud emergence and DNA replication normally are initiated at START in G_1 of the *S. cerevisiae* cell cycle. During prolonged incubation with benzimidazoles, *bub2* cells acquire a second bud and begin DNA replication in the absence of nuclear division (18). One interpretation of the data has been that *BUB2* inhibits START events when anaphase is delayed (18). Our data suggest an alternative role for *BUB2*. We found that the *bub2-1* mutation diminishes the mitotic delay induced by a dicentric chromosome and that these dicentric *bub2* cells could undergo nuclear division and cytokinesis. We did not detect a significant percentage of cells that had more than one bud or that had acquired a rereplicated genome. One possible reason for the difference in bypass phenotypes is that a spindle is present in dicentric cells but absent in cells treated with a high concentration of a benzimidazole. We propose that when a mitotic delay is bypassed as a result of loss of *BUB2* function, cells enter anaphase and divide if they have a capable spindle apparatus. This model suggests that *BUB2* functions to coordinate events at mitosis and not to make START events dependent on the completion of anaphase.

The lethality of the dicentric *rad9 bub2* double mutant demonstrates that both *BUB2* and *RAD9* contribute to the mitotic delay induced by a dicentric chromosome. As shown previously, a *bub2* mutant has wild-type sensitivity to gamma irradiation (18) and a *rad9* mutant has wild-type sensitivity to benzimidazoles (45), indicating that the *RAD9*- and *BUB2*-dependent checkpoints function independently. We interpret the inviability of the dicentric double mutant to mean that while some cells successfully orient dicentric sister chromatids and divide normally, other cells encounter dicentric chromosome breaks, suffer spindle aberrations, or are confronted by both defects. Given the mechanical difficulty of segregating a dicentric chromosome on the spindle apparatus, both types of damage are expected.

Is there prometaphase in *S. cerevisiae*? Two models can account for the increased rate of loss of the dicentric chromosome in a *rad9* mutant if it is inferred that breakage results from opposing forces being applied to the two centromeres by the mitotic spindle. First, the two kinetochores of a dicentric chromatid might be attached to microtubules emanating from opposite spindle poles, thereby forming an anaphase bridge which is ultimately broken. This break might then persist through the subsequent cell cycle to arrest the cell during G_2 in a *RAD9*-dependent manner. We consider this unlikely because losses were measured in a diploid strain in which the information necessary for repairing double-strand breaks was present on the Ch. III homolog throughout the cell cycle. Previous studies have shown that the majority of recombinational DNA repair in diploids occurs during G_1 (6, 7), making it unlikely that double-strand breaks induced during anaphase would persist until G_2 of the subsequent cell cycle.

Alternatively, the chromosome break and the *RAD9*-dependent repair might occur during the same cell cycle. Dicentric chromosome breakage could occur at a stage analogous to that of prometaphase in other eukaryotic organisms. In newt lung cells, the chromosomes move during prometaphase at velocities 10 times faster than during anaphase (39). These rapid chromosome oscillations during prometaphase are thought to reflect the mechanism that establishes the bipolar orientation of sister chromatids (1, 41). A dicentric chromatid could be broken by prometaphase forces pulling the kinetochores toward opposite poles during repeated attempts at orientation. Breaks occurring at this stage in the cell cycle would be expected to activate the *RAD9*-dependent checkpoint and result in a delay of anaphase. Although the individual chromosomes of *S. cerevisiae* cannot be directly observed (11), Palmer et al. have used vital fluorescent staining and computer enhanced imaging to visualize the DNA oscillating between the mother and daughter cells (34). These nuclear transits are microtubule dependent and may represent prometaphaselike chromosome movements necessary for bipolar chromatid attachments in *S. cerevisiae*.

Mitotic events under checkpoint control in *S. cerevisiae*. The type of spindle aberration induced by a dicentric chromosome and detected by the *BUB2*-dependent checkpoint is not known. One possibility is that spindle fibers are damaged while attempting to orient the dicentric chromatid. Such an event would be significant in *S. cerevisiae* because of the paucity of spindle microtubules (35). Alternatively, a dicentric chromosome might conceivably complicate the process by which sister chromatids orient on the mitotic spindle, resulting in a delay in the cell cycle for resolving the spindle attachments of sister kinetochores. In higher eukaryotic cells, prometaphase is lengthened when bipolar orientation of a single chromosome is prevented experimentally (46) or fails to occur spontaneously (33, 39). Prometaphase cannot be extended indefinitely (46), however, implying that the consequence of perturbing chromatid orientation in higher eukaryotic cells is a delay that is under checkpoint control (15). Therefore, the *BUB2*-dependent response of *S. cerevisiae* to a dicentric chromosome might be analogous to the response of other eukaryotic cells to a chromosome that is improperly oriented on the mitotic spindle.

The delay found in diploids grown in the presence of galactose required *BUB2* but may result from a type of spindle aberration different from that of cells responding to

an activated dicentric chromosome. The conditionally repressed centromere on Ch. III might still sponsor a partial kinetochore which signals a G_2/M delay in diploids. Hill and Bloom (17) demonstrated that a protein complex remains associated with *GALCEN3* while transcription is induced from the *GAL1* promoter. This complex possibly retains partial kinetochore function, since the conditional dicentric chromosome was lost during growth in the presence of galactose at a frequency 1,000-fold greater than for a monocentric chromosome. Diploids may be affected more than haploids by a partially repressed *GALCEN3* centromere because of a greater sensitivity to segregational perturbations. Diploids are more sensitive to benomyl (44) and can tolerate fewer copies of centromere-containing plasmids (38) than can haploids. *BUB2* function might therefore be involved in the cellular response to an improperly oriented chromosome (i.e., a dicentric chromosome), a defective kinetochore, or both.

We have characterized a delay in the cell division cycle that may be similar to other mitotic delays that have been described for *S. cerevisiae*. Cells segregating a minichromosome with a mutant centromere sequence (43), some strains grown under selection for an excess number of centromere plasmids (8, 38), and a mutant with a high rate of chromosome loss (9) all exhibited a late cell cycle delay. In each case, the process of chromosome segregation was presumably perturbed by interfering with centromere function. The involvement of mitotic checkpoints in these events will provide a more thorough understanding of the mechanisms which integrate spindle and centromere function with other events in the cell division cycle.

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